

# Flavivirus serosurveys in dogs, horses and wild boar, the Netherlands 2018-2022

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## Flaviviruses in the Netherlands

Flaviviruses are arthropod-borne viruses (*arboviruses*) from the Flaviviridae family.

USUV caused large die-offs in birds in 2016 and has been circulating since. TBEV is also present in the Netherlands, and WNV has been detected in birds and mosquitoes in 2020 and again in one bird in 2022. In this study, we aim to determine the baseline seroprevalence for three flaviviruses: West Nile Virus (WNV), Usutu Virus (USUV) and Tick-Borne Encephalitis Virus (TBEV) in horses, dogs and wild boar (*Sus scrofa*) in the Netherlands.

## Materials and methods

### Horses and dogs

- Serum was collected by veterinarians throughout NL
- Sampling period May 2021 – May 2022
- A short survey was filled out by horse and dog owners, with questions on demographics and insect bite prevention measures

### Wild boar

- Serum was collected by hunters from 2018 until 2020
- Sampling was part of ongoing surveillance program wild boar for ASF, CSF etc.

### Serology

- Sera were first screened for IgM/IgG using commercial multi-species ELISA (IDVet, WNV competition ELISA)
- Doubtful and positive samples were tested by virus neutralization tests (VNTs) for TBEV, USUV and WNV
- Equine ELISA positive samples were also tested for IgM

## Results

### Wild boar

- ELISA results are shown in table 2.
- VNTs have been performed for ELISA positive samples from 2020 and will be performed for samples from 2018, 2019 and 2021 as well.

Year	ELISA positive/tested (%)
2018	167/372 (44.89%)
2019	123/390 (31.53%)
2020	91/354 (24.52%)
2021	80/388 (20.62%)

Table 2. Wild boar ELISA results

- For samples from 2020, 8/(91), 4/(91) and 3/(91) ELISA positives were confirmed as respectively TBEV, USUV and WNV infections when using calculations as described in literature ( $\geq 4$ -fold higher titer for one virus compared to the others)
- For 34 samples which were positive in one or more VNTs, the definite infecting virus could not be determined

### Plans

- Use a modelling approach to estimate probabilities of infection for specific flaviviruses for each animal, based on the available ELISA and VNT outcomes
- Hereby taking into account cross-reactions and co-circulation
- Investigate possible correlation between ELISA outcome and VNT titres
- Estimate seroprevalence of investigated flaviviruses based on the model outcomes

## Results

### Horses and dogs

- Serology results are shown in figure 1
- All horses were negative for WNV IgM
- For horses, a seroprevalence of **0.27% (95% CI [0.00-0.81])** was found for both WNV and TBEV
- One dog was USUV positive, resulting in seroprevalence of **0.39% (95% CI [0.00-1.15])**
- Owner questionnaire results are shown in table 1

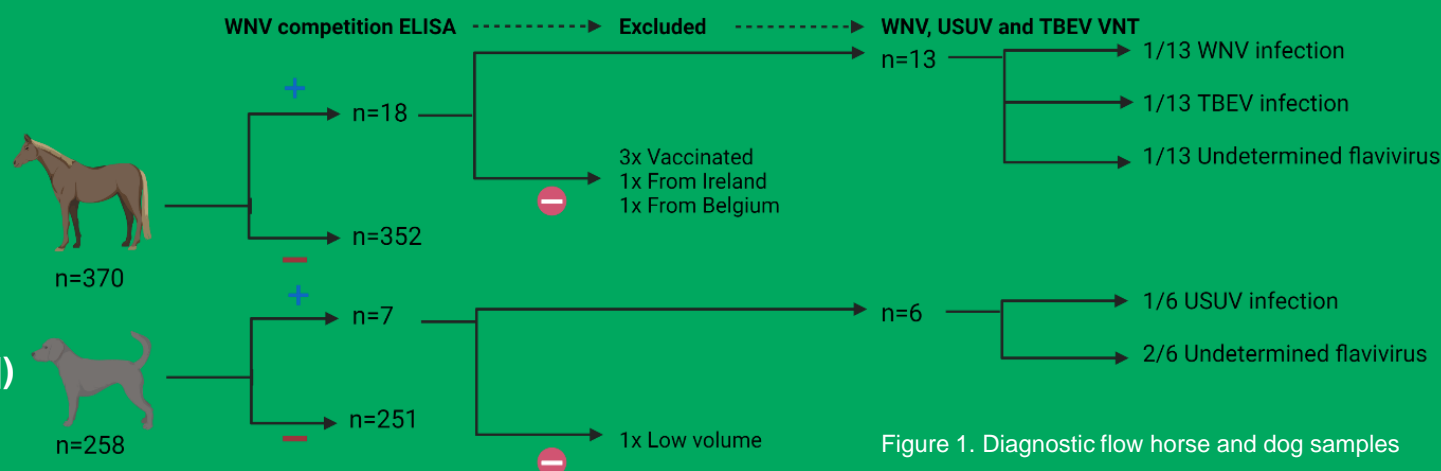


Figure 1. Diagnostic flow horse and dog samples

Table 1. Owner questionnaire results. <sup>1</sup> Median (IQR); n (%)

	Species	
	Horse, N = 364 <sup>1</sup>	Dog, N = 258 <sup>1</sup>
<b>Age</b>	12 (6, 17)	4 (1, 8)
<b>Sex</b>		
Female	196 (54%)	139 (54%)
Male	168 (46%)	119 (46%)
<b>Housing area</b>		
Agricultural	273 (75%)	43 (17%)
Agricultural/Nature	8 (2.2%)	2 (0.8%)
Urban	51 (14%)	204 (79%)
Urban/Agricultural	3 (0.8%)	3 (1.2%)
Nature	28 (7.7%)	5 (1.9%)
<b>Amount of time spent outside in 24h</b>		
0-6 hours	92 (25%)	203 (79%)
6-12 hours	139 (38%)	40 (16%)
12-18 hours	30 (8.2%)	7 (2.7%)
>18 hours	103 (28%)	6 (2.3%)
<b>Use of repellent in vector season</b>		
No	236 (65%)	88 (35%)
Yes	128 (35%)	166 (65%)
<b>Tick(s) removed within 12 months prior to sampling</b>		
No	328 (90%)	171 (67%)
Yes	35 (9.6%)	83 (33%)

## Discussion

- Cross-reactivity and co-circulation complicate interpretation of flavivirus serology
- Seroprevalences in dogs and horses are lower than expected
- The seroprevalence of flaviviruses in wild boar appear higher than expected, but more tests and analyses must be performed
- Sampling of wild boar is restricted to specific regions and readout of tests are restricted by sample quality – which results in bias
- Wild boar might be a better species to target for flavivirus early warning compared to horses and dogs? – more research and testing needed to confirm this hypothesis

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